REMARKS

I. PRELIMINARY COMMENTS

The present invention is directed to specific binding assays in which the presence of an analyte in a sample is assayed for by steps including reaction of a specific binding partner for the analyte with the analyte to form a specific binding partner-analyte complex and detection of that complex. (One example of a specific binding partner-analyte complex is the complex formed between an antigen and an antibody specific for that antigen.) More particularly, the method of the invention includes the steps of mixing a sample of cells with a cell lysis agent to provide a lysed cellular sample, mixing the lysed cellular sample with a cyclodextrin sequestrant for the cell lysis reagent, and performing the specific binding assay in the presence of that sequestrant.

Claims 1 and 18 have been amended to incorporate the suggestions made by the Examiner in her letter of 22 May 2001. In particular, Claim 1 has been amended to recite the presence of a tracer and claim 18 has been amended to clarify the subject matter of that claim. These amendments do not introduce new matter into the application and are supported in the specification such as at: page 5, line 26; page 14, lines 28-29; page 48, lines 25-28. Claim 15 has been deleted in response to the Examiner's objection that it is a substantial duplicate of Claim 10.

II. OUTSTANDING REJECTIONS

Claim 15 stands objected to as being a substantial duplicate of claim 10.

Claims 1-2, 4-13, 15-19 and Claim 21 stand rejected under 35 U.S.C. §112 (second paragraph) as being indefinite.

Claims 14 and 20 stand rejected under 35 U.S.C. §102(e) over Lundin, U.S.Patent No. 5,705,345.

Claims 1-2, 4-5, 8, 10, 14-18 and 20 stand rejected under 35 U.S.C. §102(b) over Khanna, U.S. Patent No. 5,032,503.

Claims 7, 11-13 and 19 stand rejected under 35 U.S.C. §103(a) over Khanna, U.S. Patent No. 5,032,503 in view of Brown et al., U.S. Patent No. 5,739,001.

Claims 6 and 9 stand rejected under 35 U.S.C. §103(a) over Khanna, U.S. Patent No. 5,032,503 in view of Cook WO 94/26413.

Claim 21 stands rejected under 35 U.S.C. §103(a) over Khanna, U.S. Patent No. 5,032,503 in view of Edmonds, U.S. Patent No. 6,159,750.

III. PATENTABILITY ARGUMENTS

A. The Rejection Under 35 U.S.C. §112 (Second Paragraph) Should Be Withdrawn

The rejection under 35 U.S.C. §112 (second paragraph) should be withdrawn in light of the amendment of the claims.

B. The Rejection of Claims 14 and 20 Under 35 U.S.C. §102(e) Over Lundin Should Be Withdrawn

The rejection of claims 14 and 20 under 35 U.S.C §102 (e) as being anticipated by Lundin U.S. Patent No. 5,705,345 should be withdrawn because Lundin is not directed to specific binding assays. Instead, Lundin discloses a kit (col. 7, lines 1-9) comprising a) an extracting substance; b) a cyclodextrin; c) a firefly luciferase reagent, and d) an assay buffer. Thus, the assay described by Lundin does not involve specific binding reactions; rather the intracellular component to be measured (ATP) acts as a co-factor for the luciferase enzyme-catalysed reaction with luciferin to produce light. Thus the "firefly

luciferase reagent" disclosed by Lundin is not a "specific binding partner for the analyte" but a mixture of enzyme and substrate. In contrast, the essential features of claim 20 comprise a detergent, a cyclodextrin sequestrant for the detergent, and a specific binding partner for the analyte.

Lundin also discloses a "kit for nucleic acid manipulation" which comprises an extractant, a cyclodextrin for neutralising the extractant, and at least one polymerase or restriction enzyme for further processing the recovered nucleic acid by amplification or enzymic modification or restriction. The polymerase or restriction enzymes are not "specific binding partners" such as an antibody as recited by the present claims, but rather are used for "further processing" of the nucleic acid. The kit of Claim 20, therefore, is not anticipated by either of the kits disclosed by Lundin.

While, with respect to Claim 14, the Examiner asserts that Lundin discloses a kit suitable for lysis and assay of analytes comprising a lysis reagent (surfactant), cyclodextrin, specific binding partner, i.e.firefly luciferase reagents, an assay buffer and a separation means (i.e. dipstick) for separating bound tracer from unbound tracer it does not describe a kit having a "specific binding partner" for the reasons discussed above. Moreover, Lundin does not disclose either a "tracer" or "separation means for separating bound tracer from unbound tracer" which are essential features of the claimed invention. The detection method used in the Lundin assay is the production of light in the luciferase reaction with luciferin, and does not involve the use of a "tracer". Furthermore, the "dipstick" separation means referred to by Lundin is simply a means for "carrying dried reagents for the removal of non-microbial ATP and of sucking up a defined volume of urine" (ATP Luminescence: Rapid Methods in Microbiology, P.E.Stanley et al. (Eds.), Blackwell

Scientific Publications, 1989, page 25, second paragraph), and not a means of separating bound tracer from unbound tracer.

The "kit for nucleic acid manipulation" disclosed in Lundin also lacks many of the essential features of the kit of claim 14 because that kit lacks "specific binding partner" for the analyte (as discussed above), a tracer, and separation means for separating bound tracer from unbound tracer. For these reasons, the rejection of claims 14 and 20 over Lundin should be withdrawn.

C. The Rejection Under 35 U.S.C. §102(b) Over Khanna Should Be Withdrawn

The rejection of claims 1-2, 4-5, 8, 10, 14-18, and 20 under 35 U.S.C. §102(b) by Khanna (U.S. Patent No. 5,032,503) should be withdrawn because Khanna does not disclose mixing a sample of cells with a cell lysis reagent, but rather the use of a surfactant to inhibit complex formation between complementary members of a specific binding pair (column 4, lines 5-8), specifically the two components of the enzyme β-galactosidase. In particular, Khanna discloses a formulation method for a component of an assay and, notably, a method for keeping normally interacting components of a specific binding pair apart in a single liquid reagent. The present invention, conversely, claims the step of mixing a sample of cells with a cell lysis reagent (e.g. a detergent) as a sample pre-treatment method before assaying for an analyte. In fact, with the assays described in Khanna, the purpose of the addition of a detergent is to dissociate a complex between the complementary members of a specific enzyme, β-galactosidase, which functions in the assay to generate a signal. This step takes place to form a test reagent in a single liquid prior to introducing that liquid single reagent to the sample. As discussed at column 3, lines 11 to 20 of Khanna, the liquid single reagent may be used in an assay method that can be used with any aqueous sample containing the analyte. It is further stated that "sample pre-

treatment will follow conventional procedures" (page 3, lines 19-20), and 'in the case of physiological fluids, other than the removal of particulates, no pre-treatment of the sample will usually be performed for purposes of the instant assay method' (column 5, lines 43-46).

It is thus clear that Khanna not only fails to disclose the step of mixing a sample of cells with a detergent prior to assaying, but it actually teaches away from such a step. Khanna therefore fails to disclose each and every element of the claimed invention and the anticipation rejections of claims 1, 2, 4, 5, 8, 10, 14-18, and 20 should be withdrawn.

D. The Rejection Under 35 U.S.C. §102(a) Over Khanna In View of Brown Should Be Withdrawn

The rejection of claims 7, 11-13 and 19 under 35 U.S.C. §103(a) as being unpatentable over Khanna in view of Brown, U.S. Patent No. 5,739,001 should be withdrawn because Brown should not properly be combined with Khanna and does not make up for the deficiencies in Khanna, even if they were combined. Specifically, Brown discloses a "solid phase one-well cell based assay" which allows the study of biochemical processes in living cells responding to a stimulus by evaluating cell-related analytes (i) without the need to attach cells to the solid phase, (ii) without the need to employ cell culture techniques, and (iii) without the need to radioactively label the cell (column 3, lines 46-51). The assay disclosed in Brown involves lysis of the cell with a detergent (e.g. Triton X-100) but does not involve sequestration of the cell lysis reagent with a cyclodextrin. In fact, Brown is completely silent with regard to any adverse effects that the cell lysis reagent (e.g. Triton X-100) may have upon the binding reaction of the analyte to its specific binding partner.

As discussed above, Khanna describes the use of a surfactant to inhibit complex formation between complementary members of a specific binding pair (and not as a cell lysis reagent), and the addition of a cyclodextrin to neutralise the surfactant and thus initiate complex formation. The concentrations of surfactant cited in Khanna to inhibit binding range from 0.2-1.0% (column 4 lines 13-19) and from 10-30% (column 5, lines 9-11). In contrast, the highest concentrations of surfactant used in the 'detection mix' of Brown (column 7, lines 1-12), prior to dilution in the cell/stimulation media, is only 0.35%.

It is submitted that there is no motivation for the skilled person to combine the teachings of Brown with those of Khanna because they relate to different and unrelated concepts (i.e. cell lysis versus keeping binding partners apart). Furthermore, the skilled person would not be led to even consider the problems caused by the use of surfactants in the assay of Brown by the teachings of Khanna because of the much higher concentrations used therein. Therefore the skilled person would have no motivation to contemplate the use of cyclodextrin in the assay of Brown to overcome the problems addressed by the present invention. Accordingly, the rejection of claims 7, 11-13, and 19 under 35 U.S.C. §103(a) should be withdrawn.

E. The Rejection of Claims 6 and 9 Under 35 U.S.C. §103(a) Over Khanna and Lock Should Be Withdrawn

The rejection of claims 6 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Khanna in view of Cook (WO 94/26413) should be withdrawn because the person skilled in the art would not be motivated to incorporate the multiwell system described in Cook into the present invention. Cook discloses an apparatus and method for studying cellular processes in intact cells using scintillation proximity assays. The apparatus comprises a vessel having a scintillant base which is adapted for the growth and

attachment of cells. The method comprises introducing into a fluid suspension of cells a radiolabelled reagent under conditions to cause a portion of the labelled reagent to become associated with or released from the cells in order to study the cellular process.

Specifically, an essential feature of the present invention is the ability to lyse cells within the assay dish, then utilize cyclodextrin to neutralize the cell lysis reagent to enhance SPA specific binding reactions. The main focus of the multiwell system in Cook is that the scintillant incorporated into the plastic allows the cells to remain intact. As a result, Cook teaches away from the need to disrupt cells in order to measure cellular analytes.

As discussed above, Khanna does not describe a pre-treatment lysis method. Furthermore, neither Cook nor Khanna teach lysis of cells with a detergent. Thus the combination of Khanna with Cook would not lead the skilled person to the present invention where cells are cultured, lysed and assayed in a single reaction vessel.

F. The Rejection of Claim 21 Under 35 U.S.C. §103(a) Over Khanna and Edmonds Should Be Withdrawn

The rejection of claim 21 over Khanna in view of Edmonds U.S. Patent No. 6,159,750 should be withdrawn. Edmonds teaches a method of fluorescence polarisation immunoassay which involves detecting and measuring the concentration of an analyte in a sample. Nowhere within the patent is there any disclosure of a sample pre-treatment to lyse intact cells prior to the determination of the analyte concentration. Furthermore, the patent is completely silent with regards to the use of any sample pre-treatment, other than "diluents or analytical reagents" (column 4, lines 18-19). In fact, although there is reference to the sample being "a body fluid such as blood, serum, plasma, or urine etc." (column 4, lines 35-36), there is no mention of the method being applicable for use in "a cell sample." Accordingly, Edmonds fails to make up for the deficiencies in Khanna which

also fails to disclose the step of mixing a sample of cells with a cell lysis reagent prior to assaying. Thus a combination of the teachings of Edmonds with those of Khanna would not lead the skilled person to arrive at the present invention, which is inventive thereover and claim 21 should be allowed.

CONCLUSION

For the foregoing reasons, the rejections should be withdrawn and all claims 1, 2, 4-14, and 16-21 should be allowed. Should the Examiner wish to discuss any issues of form or substance in order to expedite allowance of the pending application, she is invited to contact the undersigned attorney at the number indicated below.

Attached hereto are the changes made to the claims by the current amendments. The attached page is captioned "Version with Markings to Show Changes Made."

The Commissioner is authorized to charge any fee deficiency required by this paper to Deposit Account No. 13-2855.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claim 15 has been cancelled.

Claims 1 and 18 have been amended as follows:

1. [FOUR TIMES AMENDED] A method of conducting a specific binding assay for the presence of an analyte in a cell sample which method comprises the steps of:

i) mixing a sample of cells with a cell lysis reagent to provide a lysed cellular sample;

ii) mixing the lysed cellular sample with a specific binding assay reagent comprising a specific binding partner of the analyte and a tracer to perform a specific binding assay by forming a reaction mixture comprising a specific binding partner –analyte complex;

iii) mixing the lysed cellular sample with a cyclodextrin sequestrant for the cell lysis reagent, whereby the specific binding assay of step ii) is performed in the presence of the sequestrant; and

iv) detecting the presence of the specific binding partner-analyte complex the presence of which is indicative of the presence of the analyte in the sample.

18. [TWICE AMENDED] The method as claimed in claim 1, wherein the assay reagent further comprises a label for detection wherein the label is [specific binding partner is labelled with a label] selected from the group consisting of radioactive isotope labels, enzyme-linked labels and fluorescent labels.